Check List the journal of biodiversity data

NOTES ON GEOGRAPHIC DISTRIBUTION

Check List 17 (1): 115–123
https://doi.org/10.15560/17.1.115

PENSOFT

New records of the troglobitic *Hyalella veredae* Cardoso & Bueno, 2014 (Crustacea, Amphipoda, Hyalellidae) from Minas Gerais caves, southeast Brazil, with notes on its natural history

Tamires Zepon[1*], Leonardo P. A. Resende[2], Alessandra A. P. Bueno[3], Maria Elina Bichuette[1]

1 Laboratório de Estudos Subterrâneos, Departamento de Ecologia e Biologia Evolutiva, Universidade Federal de São Carlos, São Carlos, SP, Brazil • TZ: tazepon@gmail.com https://orcid.org/0000-0001-7356-8631 • MEB: lina.cave@gmail.com https://orcid.org/0000-0002-9515-4832

2 Núcleo de Etologia e Evolução, Instituto de Biologia, Universidade Federal da Bahia, Salvador, BA, Brazil • biologo.leonardoparesende@gmail.com https://orcid.org/0000-0001-5386-7618

3 Departamento de Ecologia e Conservação, Universidade Federal de Lavras, Lavras, MG, Brazil • aapbueno@yahoo.com.br https://orcid.org/0000-0001-7809-7189

* Corresponding author, tazepon@gmail.com

Abstract

Since its description, the troglobitic amphipod *Hyalella veredae* Cardoso & Bueno, 2014 has been recorded only in its type locality, Vereda da Palha cave, in southeastern Brazil. We report this species from three other caves in the region, expanding its distribution by at least 20 km². Inter- and intra-population variability in eye size was observed. Two caves had larger populations, probably due to the presence of biofilm. The species was classified as Critically Endangered due to its restricted area of occurrence, and regional impacts and threats.

Keywords

Biofilm, conservation, endangered species, hypogean streams, morphological variation, subterranean environment, troglobite

Academic editor: Jesser Fidelis de Souza Filho | Received 25 September 2020 | Accepted 2 January 2021 | Published 18 January 2021

Citation: Zepon T, Resende LPA, Bueno AAP, Bichuette ME (2021) New records of the troglobitic *Hyalella veredae* Cardoso & Bueno, 2014 (Crustacea, Amphipoda, Hyalellidae) from Minas Gerais caves, southeast Brazil, with notes on its natural history. Check List 17 (1): 115–0123. https://doi.org/10.15560/17.1.115

Introduction

Amphipods (Malacostraca) are an order of macroscopic crustaceans with most species inhabiting marine ecosystems, but several species occur in various freshwater habitats, and few species are terrestrial (Väinölä et al. 2008). Amphipods play a substantial role in aquatic food webs, as they can be important in the diets of fish, and have diverse feeding habits, such as herbivory, detritivory, carnivory, or omnivory (Väinölä et al. 2008). According to Rogers et al. (2020), 156 species, 47 genera in 20 families of freshwater or slightly brackish water amphipods from inland waters are recognized in the Neotropical region.

Amphipods are diverse in the subterranean environment, with approximately 45% of all freshwater species being troglobitic (Väinölä et al. 2008), i.e., presenting source populations that are exclusively subterranean

(Trajano 2012). Troglobitic species usually have troglomorphisms, such as loss of eyes and melanic pigmentation, as well as elongation of the trunk and/or appendages (Väinölä et al. 2008). Most subterranean species are omnivorous and dependent on organic debris from an epigean environment (Väinölä et al. 2008).

There are 15 known troglobitic species of amphipods in Brazilian caves: one Mesogammaridae (*Potiberaba porakuara* Fišer, Zagmajster & Ferreira, 2013) and one Seborgiidae (*Seborgia potiguar* Fišer, Zagmajster & Ferreira, 2013), both from the state of Rio Grande do Norte; one Bogidiellidae (*Megagidiella azul* Koenemann & Holsinger, 1999) from the state of Mato Grosso do Sul; seven species of Artesiidae (*Spelaeogammarus bahiensis* Brum, 1975; *S. santanensis* Koenemann & Holsinger, 2000; *S. spinilacertus* Koenemann & Holsinger, 2000; *S. trajanoae* Koenemann & Holsinger, 2000; *S. titan*, Senna, Andrade, Castelo-Branco & Ferreira, 2014; *S. sanctus* Bastos-Pereira & Ferreira, 2015) from the state of Bahia, and *S. uai* Bastos-Pereira & Ferreira, 2017 from the state of Minas Gerais. Also, there are six species of Hyalellidae.

The family Hyalellidae is composed only of *Hyalella* Smith, 1874, and it probably originated in South America (Väinölä et al. 2008). The genus has a distribution restricted to Nearctic and Neotropical regions (Väinölä et al. 2008), and 83 species have been described (Horton et al. 2020), of which 30 occur in Brazil (Reis et al. 2020).

Ten hypogean species of *Hyalella* were described. *Hyalella muerta* Baldinger, 2000 occurs in a tunnel in Death Valley National Park, California, USA (Baldinger et al. 2000). *Hyalella cenotensis* Marrón-Becerra, Hermoso-Salazar & Solís-Weiss, 2014 and *H. maya* Marrón-Becerra, Hermoso-Salazar & Solís-Weiss, 2018 occur both from a sinkhole (cenote) in Mexico (Marrón-Becerra et al. 2014, 2018). *Hyalella anophthalma* Ruffo, 1957 is endemic to a cave (Cueva de Rio Gueque) in Venezuela (Ruffo 1957), and *H. imbya* Rodrigues & Bueno, 2012 occurs in a hypothelminorheic habitat in Brazil (Rodrigues et al. 2012). Five species occur in caves in Brazil: *H. caeca* Pereira, 1989, *H. formosa* Cardoso & Araujo, 2014, *H. epikarstica* Rodrigues, Bueno & Ferreira, 2014, *H. spelaea* Bueno & Cardoso, 2011, and *H. veredae* Cardoso & Bueno, 2014 (Pereira 1989; Cardoso et al. 2011, 2014; Rodrigues et al. 2014).

All Brazilian troglobitic *Hyalella* associated with caves are currently known only from their type locality: *H. caeca* at Tobias de Baixo cave (Iporanga municipality) (Pereira 1989), *H. formosa* at Andorinhas Cave (Ponta Grossa) (Cardoso et al. 2014), *H. epikarstica* at Areias de Cima cave (Iporanga) (Rodrigues et al. 2014), *H. spelaea* at Toca cave (Itirapina) (Cardoso et al. 2011), and *H. veredae* at Vereda da Palha cave (Presidente Olegário) (Cardoso et al. 2014).

Cave amphipods present variation in their morphological characters, which is related to their isolation in subterranean environments (Culver et al. 1995; Fišer et al. 2012; Delić et al. 2016; Zakšek et al. 2019). Some genera are eyeless and restricted to caves (e.g., *Accubogammarus* Karaman, 1974 and *Zenkevitchia* Birstein, 1940), while others have varying levels of eye reduction, such as the genus *Gammarus* Fabricius, 1775, and most cave species have not completely lost their eyes (Culver et al. 1995). Most hypogean species of *Hyalella* are eyeless, but there are species with eyes well-developed (*H. maya*) or reduced (*H. spelaea*), and only *H. veredae* has intraspecific variation in the eye size (Cardoso et al. 2011, 2014; Marrón-Becerra et al. 2014, 2018).

We present new data on the distribution of the troglobitic amphipod *Hyalella veredae* and include new information on its natural history and its habitat. We also discuss its conservation status and document the anthropic impacts and threats within the occurrence area of this species.

Methods

Study area. The municipality of Presidente Olegário (18°25′04″S, 046°25′04″W at approximate center) is located in the southern part of the Upper São Francisco hydrographic basin, a sub-basin of the Paracatu River, northwestern Minas Gerais, Brazil (Fig. 1A, B). The vegetation is composed of the Cerrado (savanna-like vegetation) interspersed with dry forests on limestone outcrops (Ab'Saber 1977). The climate is sub-warm and semi-humid tropical, with a dry season lasting four to five months (Nimer 1989). The limestone outcrops at Presidente Olegário belong to the Bambuí geomorphological unit (Auler et al. 2001) (Fig. 1B, C).

The municipality of Presidente Olégário is characterized by human occupation, with natural landscapes almost completely suppressed and replaced by pastures and large areas of extensive agriculture (Secutti and Bichuette 2013). Additionally, gas extraction and hydroelectric projects are being implemented (Secutti and Bichuette 2013; Zepon and Bichuette 2017). Several of these directly impact the caves and their surroundings (Zepon and Bichuette 2017) (Table 1; Fig. 2A, D).

We explored four limestone caves with water bodies inside: Vereda da Palha cave (VP), Fazenda São Bernardo cave (SB), Zé de Sidinei cave (ZS), and Toca do Charco cave (TC) (Fig. 1C). Their main characteristics are shown in Table 1. For more detailed descriptions, see Resende et al. (2016).

Collection. We visited the region on five occasions during wet and dry seasons: September 2013 (end of dry season), January 2014 (rainy season), April 2014 (beginning of dry season), June 2014 (dry season), and September 2014 (end of dry season). We searched all available bodies of water in each cave. In VP there is an epigean (= superficial) stream penetrating the cave entrance (sinkhole) and a hypogean (= subterranean) stream that flows over travertine dams in the upper level of the cave. In SB there is a hypogean stream, and in some places, small dams have formed. In ZS there is a small hypogean

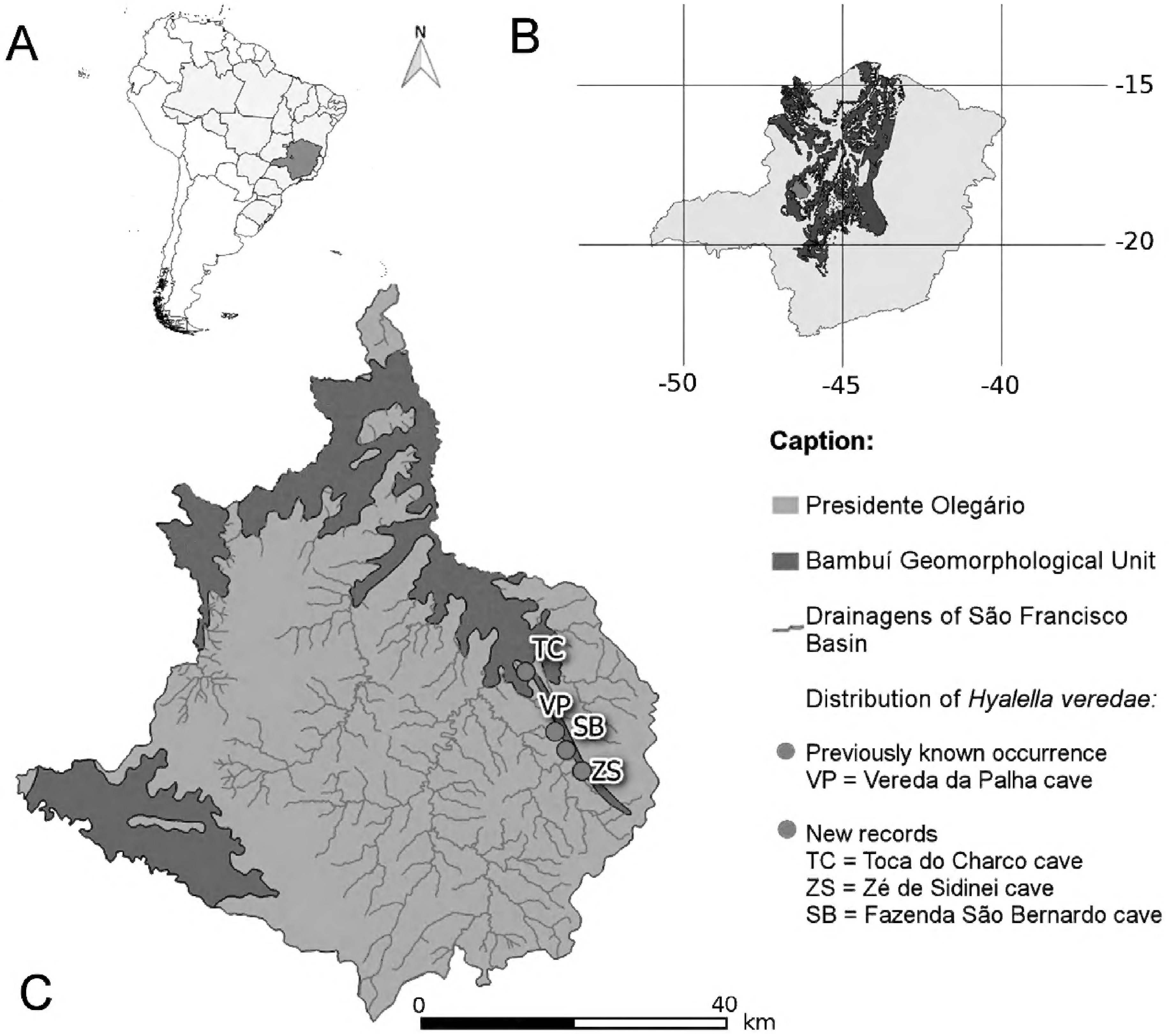

Figure 1. A. South America map indicating the position of Presidente Olegário karst area, state of Minas Gerais (dark grey shading), Brazil (light grey shading). **B.** State of Minas Gerais and the locality of the Presidente Olegário karst area (red). **C.** Presidente Olegário municipality and caves with records of *Hyalella veredae* Cardoso & Bueno, 2014.

Table 1. Characteristics of caves in Presidente Olegário, state of Minas Gerais, Brazil, with records of *Hyalella veredae* Cardoso & Bueno, 2014.

Cave	Latitude	Longitude	Mapped passageways (m)	Epigean drainage	Hypogean drainage	Impacts on its surroundings
Vereda da Palha (VP)	18°15′19″S	046°07′34″W	2,500	Present	Present	Impacted native vegetation for cattle grazing
Fazenda São Bernardo (SB)	18°16′37″S	046°06′46″W	2,000	Absent	Present	Deforested for cattle grazing; use of pesticides for agriculture
Zé de Sidinei (ZS)	18°18′06″S	046°05′41″W	650	Absent	Present	Impacted native vegetation for cattle grazing
Toca do Charco (TC)	18°11′06″S	046°09′39″W	80	Absent	Present	Impacted native vegetation for cattle grazing

stream, and in TC logged phreatic water forms a small lake (Fig. 3A–D).

Collections were made with hand nets, and most specimens were preserved in loco in 70% ethanol. Some specimens were preserved in 100% ethanol. Where the abundance of individuals was high, some specimens were collected, and the remaining were counted to avoid excessive euthanasia of specimens. The specimens were collected with permission from the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio; no. 28992-7). The examined material is deposited at the Zoological Collection of Laboratório de Estudos Subterrâneos (LES), Universidade Federal de São Carlos (UFSCar), São Carlos, Brazil (M.E. Bichuette curator).

To recognize the minimal extent of occurrence (IUCN 2012) of *H. veredae*, we drew a polygon encompassing the points of the caves using Image J software (Rueden et al. 2017). Information on the natural history of the species consisted of counting the observed individuals, determining their sex and developmental stage when collected, and describing the activity of the observed individuals (if they were swimming, hiding, or in pre-copulatory behavior). A qualitative description of the environment was also made, considering the type

Figure 2. Impacts present in Presidente Olegário karst area, state of Minas Gerais. **A–C.** Deforested surroundings of caves for grazing and agriculture practices in (**A**) Vereda da Palha cave; (**B**) Zé de Sidinei cave; (**C**) Fazenda São Bernardo cave. **D.** Garbage disposal in Fazenda São Bernardo cave. Photographs: M.E. Bichuette (A); T. Zepon (B–D).

of substrate where the individuals were found. We also verified if collected individuals of the same cave or different caves have variation in eye area size (developed, reduced, or absent eyes).

Results

Hyalella veredae **Bueno & Cardoso, 2014**
Figures 4A–C, 5A–D

Materials examined. BRAZIL – Minas Gerais • Presidente Olegário municipality, Fazenda São Bernardo cave; 18°16′37″S, 046°06′46″W; alt. ca. 800 m.; 30 Sep. 2013; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0012955, 1 adult ♂, 2 adult ♀. • same collection data as for preceding; 30 Sep. 2013; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0015136, 2 adult ♀. • same collection data as for preceding; 30 Sep. 2013; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0015137, 1 adult ♀. • same collection data as for preceding; 30 Sep. 2013; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0015138, 3 juveniles. • same collection data as for preceding; 23 Jan. 2014; T. Zepon and L.P.A. Resende leg.; LES 0012956, 1 adult ♂, 2 juveniles. • same collection data as for preceding; 13 Apr. 2014; M.E. Bichuette, T. Zepon, L.P.A. Resende, J.S. Gallo and G.F. Damasceno leg.; LES 0015147, 2 adult ♀, 1 juvenile. • same collection data as for preceding; 13 Apr. 2014; M.E. Bichuette, T. Zepon, L.P.A. Resende, J.S. Gallo and G.F. Damasceno leg.; LES 0015149, 1 adult ♂, 1 adult ♀, 3 juveniles • same collection data as for preceding; 09 Sep. 2014; T. Zepon and L.P.A. Resende leg.; LES 0015148, 2 juveniles. • same collection data as for preceding; 10 Jun. 2014; T. Zepon and L.P.A. Resende leg.; LES 0015150, 1 adult ♂ • Presidente Olegário municipality, Toca do Charco cave; 18°11′06″S, 046°09′39″W; alt. ca. 760 m; 20 Jan. 2014; T. Zepon and L.P.A. Resende leg.; LES 0012957, 5 adult ♂, 2 adult ♀. • same collection data as for preceding; 09 Sep. 2014; T. Zepon and L.P.A. Resende leg.; LES 0012960, 2 adult ♂, 2 adult ♀. • same collection data as for preceding; 09 Sep. 2014; T. Zepon and L.P.A. Resende leg.; LES 0015144, 1 juvenile. • same collection data as for preceding; 09 Sep. 2014; T. Zepon and L.P.A. Resende leg.; LES 0015152, 1 adult ♂, 1 adult ♀. • same collection data as for preceding; 09 Sep. 2014; T. Zepon and L.P.A. Resende leg.; LES 0015153, 1 adult ♂, 1 juvenile. • same collection data as for preceding; 09 Sep. 2014; T. Zepon and L.P.A. Resende leg.; LES 0015154, 4 juveniles. • same collection data as for preceding; 29

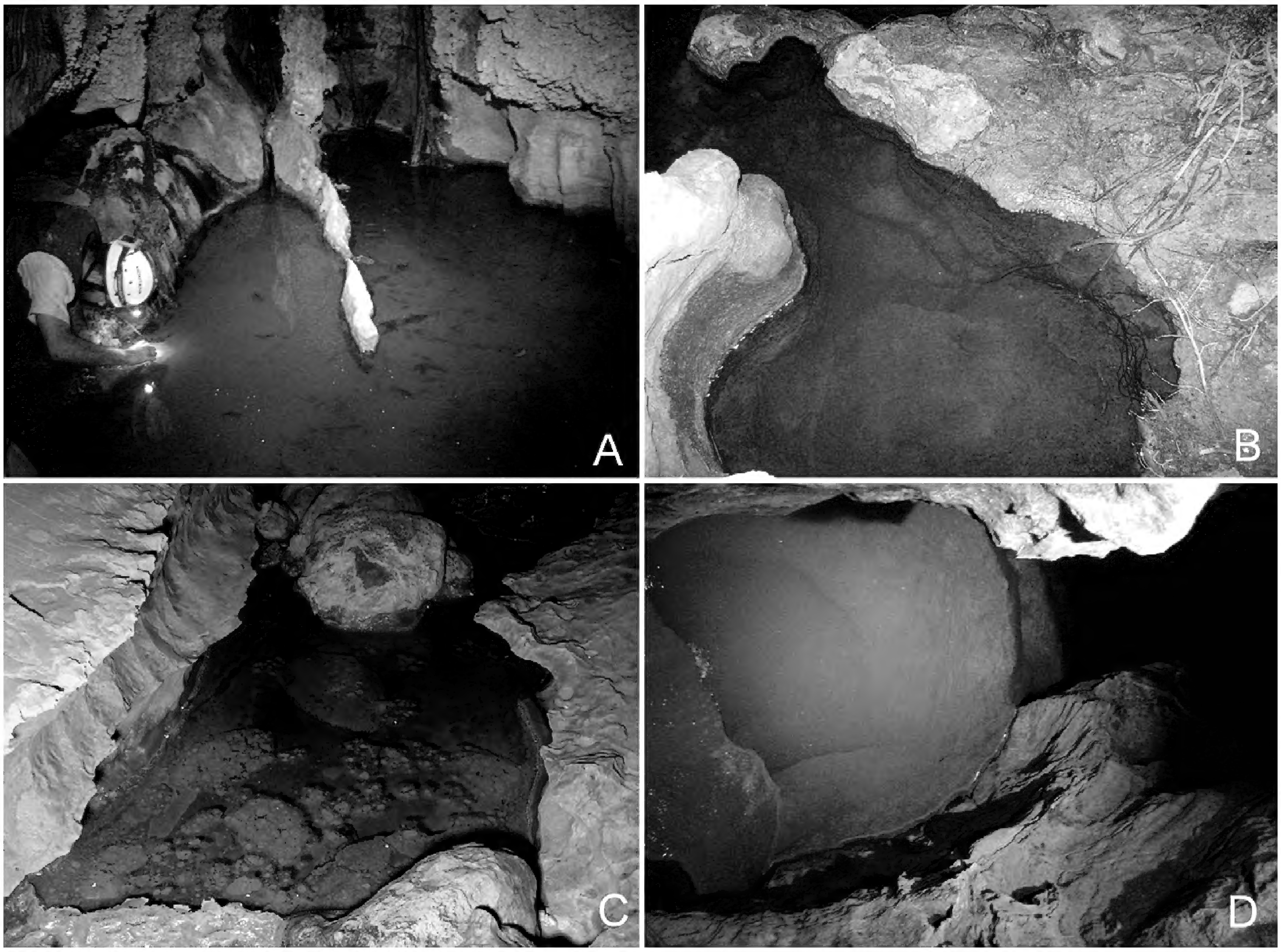

Figure 3. Collection sites of *Hyalella veredae* Cardoso & Bueno, 2014 from Presidente Olegário caves, state of Minas Gerais, Brazil. **A.** Subterranean water of Toca do Charco cave. **B.** Dam in the hypogean stream of Fazenda São Bernardo cave. **C.** Subterranean stream of Zé de Sidinei cave. **D.** Travertine dams (hypogean stream) of Vereda da Palha cave. **A, B** show the orange-colored biofilm. Photographs: T. Zepon (A, B, D); E.C. Igual (C).

Sep. 2013; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0015141, 2 adult ♂, 2 adult ♀. • same collection data as for preceding; 29 Sep. 2013; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0015142, 5 juveniles. • same collection data as for preceding; 29 Sep. 2013; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0015143, 4 adult ♂, 2 adult ♀, 1 juvenile. • same collection data as for preceding; 17 Apr. 2014; T. Zepon, L.P.A. Resende and G.F. Damasceno leg.; LES 0015146, 2 adult ♂, 1 adult ♀, 2 juveniles. • same collection data as for preceding; 13 Jun. 2014; T. Zepon and L.P.A. Resende leg.; LES 0015151, 1 adult ♂, 1 adult ♀, 1 juvenile. • Presidente Olegário municipality, Zé de Sidinei cave; 18°18′06″S, 046°05′41″W; alt. ca. 850 m; 16 Apr. 2014; T. Zepon, L.P.A. Resende and G.F. Damasceno leg.; LES 0012958, 1 adult ♂, 2 juveniles. • same collection data as for preceding; 28 Sep. 2013; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0015139, 1 juvenile. • same collection data as for preceding; 13 Jun. 2014; T. Zepon and L.P.A. Resende leg.; LES 0015140, 1 juvenile. • Presidente Olegário municipality, Vereda da Palha cave; 18°15′19″S, 046°07′34″W; alt. ca. 780 m; 12 Jun. 2014; T. Zepon and L.P.A. Resende leg.; LES 0012959, 2 adult ♂. • same collection data as for preceding; 10 Sep. 2014; M.E. Bichuette, T. Zepon, L.P.A. Resende and I.A. Ribeiro leg.; LES 0015145, 1 adult ♀.

Identification. Taxonomic treatment followed the original description of *H. veredae* (Cardoso et al. 2014) and the key to *Hyalella* species (Rogers et al. 2020). *Hyalella veredae* is characterized by a dorsally smooth body, eyes absent or reduced, antenna 1 and antenna 2 of similar size, male uropod 1 and endopod with curved setae, presence of polygonal pattern and comb scales on the carpus posterior lobe of gnathopods 1 and 2, and gnathopod 2 propodus posterior margin with comb-scales (Cardoso et al. 2014; Rogers et al. 2020) (Figs 4A–C, 5A–D).

We collected 75 individuals of *H. veredae* in the four caves, of which 25 males (33.33%), 20 females (26.66%), and 30 juveniles (40.01%). As to eye size, 19 individuals (25.33%) presented developed eyes; 51 (68%) presented reduced eyes, and five individuals (6.66%) were eyeless (Fig. 5A–D). All individuals presented the diagnostic characters of *H. veredae* (Cardoso et al. 2014).

Characteristics of habitat. We present the abundance and morphological characteristics of *H. veredae* in each cave with a brief description of the habitat.

Vereda da Palha cave. In the travertine dams formed by a hypogean stream, we found three adult specimens of

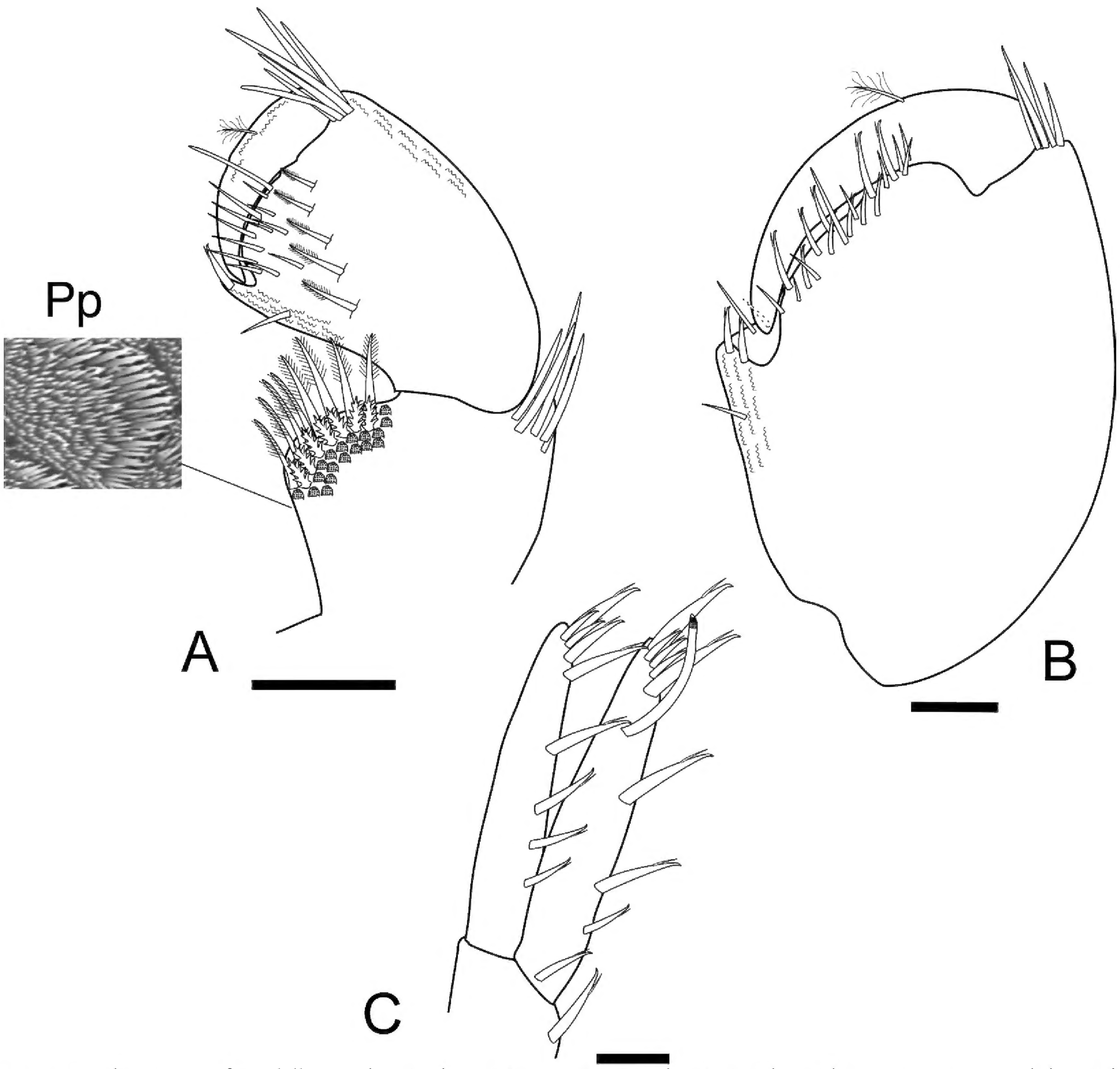

Figure 4. Diagnostic characters of *Hyalella veredae* Cardoso & Bueno, 2014 male. **A.** Gnathopod 1 carpus posterior lobe with polygonal pattern (Pp), propodus and dactylus with comb scales; **B.** Gnathopod 2 propodus with comb scales; **C.** Uropod 1 inner ramus with one curved. Scale bars: A–C = 0.1 mm. Illustrations: A.A.P. Bueno.

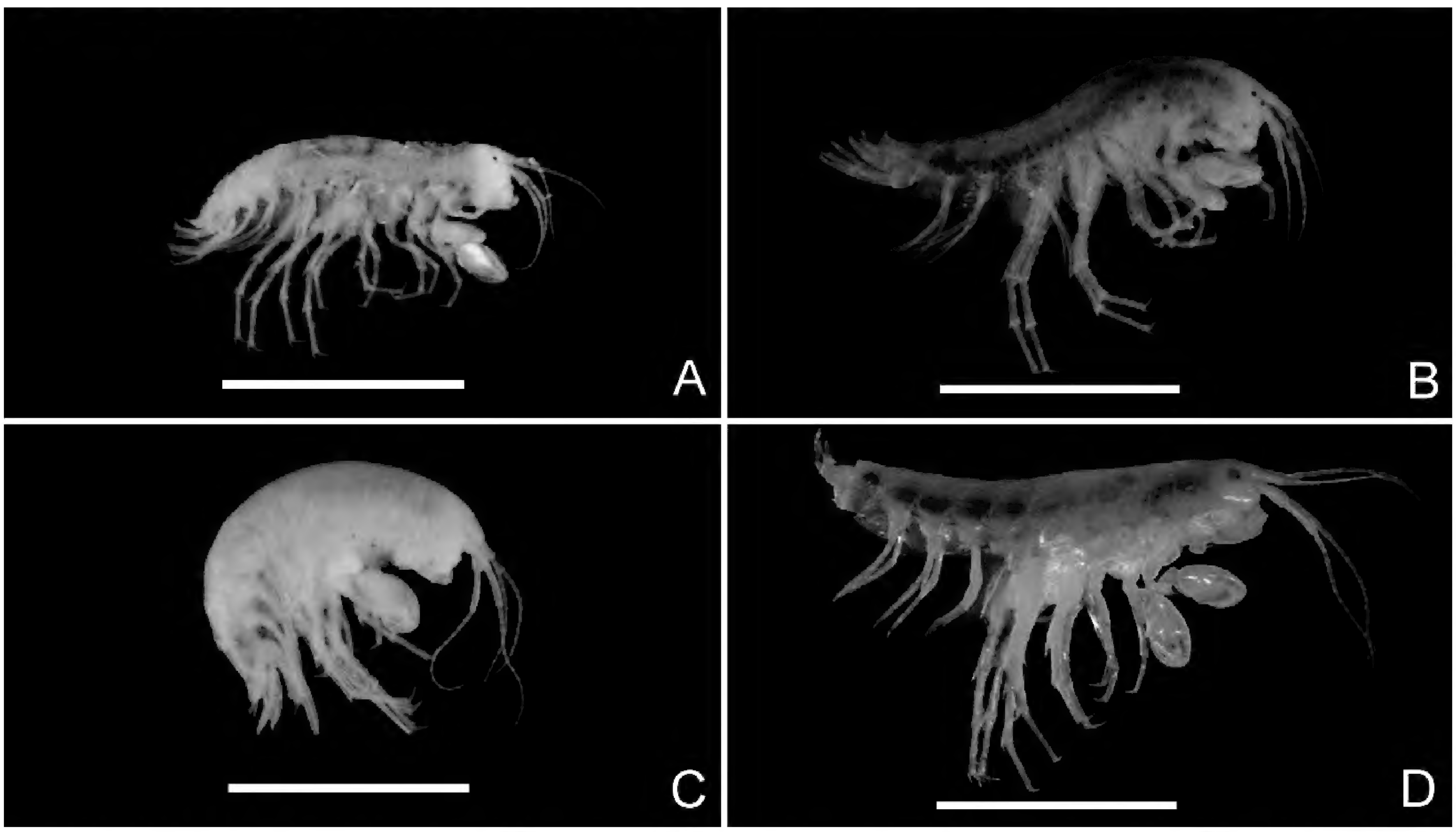

Figure 5. Male individuals of *Hyallela veredae* Cardoso & Bueno, 2014 from Presidente Olegário caves showing the similar size of antenna 1 and antenna 2 and the variation in eye size. **A.** Specimen from Vereda da Palha cave with reduced eyes. **B.** Specimen from Toca do Charco cave with reduced eyes. **C.** Specimen from Zé de Sidinei cave eyeless. **D.** Specimen from Fazenda São Bernardo cave with developed eyes. Scale bars = 3.00 mm. Photographs: T. Zepon.

H. veredae, two males and one female, all with reduced eyes (i.e., eye area size reduced about to other individuals of the same species) (Fig. 5A). The travertine is in a transition zone between twilight and aphotic (dark), and the substrate is composed of mainly clay (Fig. 3D). We observed varying water levels in travertines on sampling occasions, and in April and September 2014 some travertines were dry or experiencing lower water flows. We did not find *Hyalella* in the epigean stream.

Zé de Sidinei cave. Five individuals, one male and four juveniles, two with reduced eyes and three eyeless (Fig. 5C), were collected in the hypogean stream. The specimens were found in a lentic and shallow (ca 0.08–0.1 m) stretch of the stream, with a substrate composed mainly of clay (Fig. 3C), in a transition area between the twilight and aphotic zones of the cave. The stream level varied, and in April and September 2014 the stream was almost dry, with only a few isolated pools remaining.

Fazenda São Bernardo cave. We collected 23 individuals, eight males, eight females, and seven juveniles, of which 19 had developed eyes (Fig. 5D), three had reduced eyes, and one individual was eyeless. The specimens were found in a lentic and shallow (ca 0.20 m) stretch of hypogean stream located at the twilight zone of the cave, with a substrate composed of clay, roots, and an orange-colored biofilm (Fig. 3B). We found individuals of *H. veredae* associated with the orange-colored biofilm, which was present in all seasons. We also observed during the five visits several couples in pre-copulatory behavior. The stream level varied, with lower water flows in April and September 2014.

Toca do Charco cave. We collected 44 individuals in this cave, 18 males, 11 females, and 15 juveniles, of which 43 presented reduced eyes (Fig. 5B), and one was eyeless. In addition, we counted around 450 individuals in September 2013 and approximately 100 individuals in January and April 2014. The specimens were found in a lentic and shallow (ca 0.20–0.30 m) stretch of hypogean stream in the twilight zone of the cave, with a substrate composed of clay, roots, and an orange-colored biofilm. This biofilm was dense and abundant, occupying a significant volume of the water body, and it was present in all seasons (Fig. 3A). We also observed several couples in pre-copulatory behavior during the five visits. The stream level varied, with lower water flows in April and September 2014.

Discussion

No individual of *Hyalella veredae* was found in the epigean stream in VP cave, as observed by Cardoso et al. (2014). Our findings indicate that *H. veredae* has a large population, at least in TC cave. Our record of several couples in pre-copulatory behavior on all visits, also observed by Cardoso et al. (2014) at the beginning of the rainy season, is evidence that *H. veredae* has an established population.

The variation in the abundance of *H. veredae* in TC cave in different seasons, with fewer individuals observed during the rainy seasons, is probably related to changes in habitat, as observed in VP cave by Cardoso et al. (2014). They observed dozens of individuals of *H. veredae* in the travertines in October 2010 (beginning of the rainy season) when the water was clear, and only one specimen was found in January 2011 (rainy season) when the water was turbid, indicating habitat changes. This variation may be a response of the population to environmental conditions, as troglobites may be adapted for alternate dry and wet seasons and such adaptations may include migrations from caves to smaller spaces that are protected from extreme environmental fluctuations in the rainy season (Trajano 2013).

The high abundance of *H. veredae* in TC and SB caves may be explained by the presence of an orange-colored biofilm in these caves, as individuals of *H. veredae* are associated with it. Probably, the biofilm is utilized as a source of dissolved organic carbon and food, as observed by other authors for crustaceans, including amphipods (e.g., Covich and Thorp 2001; Simon et al. 2003). The biofilm is also used as shelter, as individuals were seen hiding within it, as observed for amphipods of the genus *Niphargus* Schiödte, 1849, which burrow in the bottoms of cave pools (Gounot 1960). The biofilm, by providing abundant shelter and food, generates a suitable microhabitat for the survival and maintenance of populations of *H. veredae*.

Amphipods lack a dispersive larval instar in their life cycle, which could reduce the potential for dispersion in contrast to other taxa with planktonic larvae (Franz and Mohamed 1989). Moreover, subterranean amphipods have limited dispersal ability (Yule 2004). Despite their limitations in dispersal, adult amphipods are good swimmers and can cover relatively long distances to find mates and places to breed (Barnard 1969). Furthermore, these animals could use subterranean water flow to disperse from one cave to another (Locke and Corey 1989; Harris et al. 2002; Persson 2001). The occurrence of *H. veredae* in four caves alongside the limestone outcrop at Presidente Olegário is evidence of a connection, at least in the past, among them. To date, there are no geological studies that indicate possible connections among these caves.

The intraspecific variation in eye size observed in *H. veredae* has not been described in other hypogean species of *Hyalella*. Among epigean species, intraspecific variation has been observed only in *H. montana* Rodrigues, Senna, Quadra & Bueno, 2017, in which anophthalmia was observed in a few individuals (Rodrigues et al. 2017). This variation in *H. veredae* may be a consequence of time and degree of isolation of each population in the subterranean environment, as suggested for the amphipod *Gammarus minus* Say, 1818 (Culver et al. 1995). As all populations show variation in eye size, and this might reflect a relatively short period of isolation in the subterranean environment, or it might indicate that these populations are still connected, which could be testable through population genetic studies.

The new records of *H. veredae* in these three caves expand its distribution area by at least 20 km^2, and makes this troglobitic *Hyalella* species the one with the largest known distribution, as the other Brazilian and non-Brazilian troglobitic species are endemic to their type locality (Ruffo 1957; Pereira 1989; Baldinger et al. 2000; Cardoso et al. 2011, 2014; Rodrigues et al. 2012, 2014; Marrón-Becerra et al. 2014, 2018).

The caves where *H. veredae* occur have high degrees of anthropogenic pressure. The existence of sinkholes (some of them with open drains), the unsaturated soil in karst systems, and the intense precipitation in tropical areas all contribute to moving contaminants more quickly through the water table and subterranean drainage (Loop 2012; Vesper 2012), mainly through water-soluble substances (e.g., fertilizers) and materials carried by water flow (e.g., solid garbage). Thus, if caves are connected by the aquifer, pollution through the subterranean systems is more pervasive.

Therefore, we propose that *H. veredae* must be classified as Critically Endangered under International Union for Conservation of Nature criteria B1ab(iii, iv, v) (IUCN 2012). The species occurs in only four caves in Presidente Olegário municipality, all within an area of 20 km^2. This area has several impacts including cattle grazing, agricultural activities, deforestation, and water pollution, and threats including projects for installation of small hydroelectric plants, and gas extraction, which contribute to declines in the quality of habitat. Declining habitat quality can infer a continued decline in the number of mature individuals and of the number of locations and subpopulations of the species.

Acknowledgements

We thank Grupo Pierre Martin de Espeleologia (GPME) for cave information, the fieldwork helpers (E.C. Igual, G.F. Damasceno, J.S. Gallo, L.A. Joaquim, and I.A. Ribeiro), the local people of Presidente Olegário for giving us free access to the caves and farms, and Jair de Sales for logistical support. We also thank the Programa de Pós-Graduação em Ecologia e Recursos Naturais (PPGERN/UFSCar) for infrastructure and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for collection permission. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq (productivity fellowships 303715/2011-1, 308557/2014-0, and 310378/2017-6 to MEB; regular project 57413/2014-0 to MEB; scholarships 132065/2013-4 and 132404/2013-3, to TZ and LPAR, respectively), and Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (2008/05678-7 and 2010/08459-4). We are grateful to Anna Jażdżewska and to an anonymous reviewer for their constructive criticisms and suggestions.

Authors' Contributions

TZ, LPAR, MEB collected the specimens and contributed for writing. AAPB identified the specimens, drew the diagnostic characters, and contributed for writing. TZ elaborated the map and formatted the figures.

References

Ab'Saber AN (1977) Os domínios morfoclimáticos na América do Sul: primeira aproximação. Geomorfologia 52: 1–22.

Auler A, Rubbioli E, Brandi R (2001) As grandes cavernas do Brasil. Grupo Bambuí de Pesquisas Espeleológicas, Belo Horizonte, Brazil, 228 pp.

Baldinger AJ, Shepard WD, Threloff DL (2000) Two new species of *Hyalella* (Crustacea: Amphipoda: Hyalellidae) from Death Valley National Park, California, USA. Proceedings of the Biological Society of Washington 113 (2): 443–457.

Barnard JL (1969) The families and genera of marine gammaridean Amphipoda. United States National Museum Bulletin 271: 1–535.

Cardoso GM, Bueno AAP, Ferreira RL (2011) A new troglobiotic species of *Hyalella* (Crustacea, Amphipoda, Dogielinotidae) from southeastern Brazil. Nauplius 19 (1): 17–26. https://doi.org/10.1590/S0104-64972011000100003

Cardoso GM, Araújo PB, Bueno AAP, Ferreira RL (2014) Two new subterranean species of *Hyalella* Smith, 1874 (Crustacea: Amphipoda: Hyalellidae) from Brazil. Zootaxa 3814 (3): 353–368. https://doi.org/10.11646/zootaxa.3814.3.3

Covich AP, Thorp JH (2001) Introduction to the subphylum Crustacea. In: Thorp JH, Covich AP (Eds.) Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, USA, 777–810.

Culver DC, Kane TC, Fong DW (1995) Adaptation and natural selection in caves: the evolution of *Gammarus minus*. Harvard University Press, Cambridge, USA, 223 pp.

Delić T, Trontelj P, Zakšek V, Fišer C (2016) Biotic and abiotic determinants of appendage length evolution in a cave amphipod. Journal of Zoology 299: 42–50. https://doi.org/10.1111/jzo.12318

Fišer C, Blejec A, Trontelj P (2012) Niche-based mechanisms operating within extreme habitats: a case study of subterranean amphipod communities. Biology Letters 8: 578–581. http://doi.org/10.1098/rsbl.2012.0125

Franz DR, Mohamed Y (1989) Short-distance dispersion in a fouling community amphipod crustacean, *Jassa marmorata* Holmes. Journal of Experimental Marine Biology and Ecology 133: 1–13. https://doi.org/10.1016/0022-0981(89)90154-8

Gounot, AM (1960) Recherches sur le limon argileux souterrain et sur son rôle nutritif pour les *Niphargus* (Amphipod gammaridés). Annales Spéléologie 15: 501–526.

Harris PM, Roosa BR, Norment L (2002) Underground dispersal by amphipods (*Crangonyx pseudogracilis*) between temporary ponds. Journal of Freshwater Ecology 17 (4): 589–594. https://doi.org/10.1080/02705060.2002.9663936

Horton T, Lowry J, De Broyer C, Bellan-Santini D, Coleman CO, Corbari L, Costello MJ, Daneliya M, Dauvin JC, Fišer C, Gasca R, Grabowski M, Guerra-García JM, Hendrycks E, Hughes L, Jaume D, Jazdzewski K, Kim YH, King R, Krapp-Schickel T, LeCroy S, Lörz AN, Mamos T, Senna AR, Serejo C, Sket B, Souza-Filho JF, Tandberg AH, Thomas JD, Thurston M, Vader W, Väinölä R, Vonk R, White K, Zeidler W (2020). World Amphipoda Database. *Hyalella* S.I. Smith, 1874. World Register of Marine Species. http://www.marinespecies.org/aphia.php?p=taxdetails&id=158104. Accessed on: 2020-09-19

IUCN (International Union for Conservation of Nature) (2012) IUCN Red List categories and criteria: version 3.1. International Union for Conservation of Nature, Gland, Switzerland, 32 pp.

Locke A, Corey S (1989) Amphipods, isopods and surface currents: a

case for passive dispersal in the Bay of Fundy, Canada. Journal of Plankton Research 11 (3): 419–430.

Loop CM (2012) Contamination of cave waters by nonaqueos phase liquids. In: White WD, Culver DC (Eds.) Encyclopedia of caves. Elsevier, Waltham, USA: 166–172. https://doi.org/10.1016/B978-0-12-814124-3.00036-4

Marrón-Becerra A, Hermoso-Salazar M, Solis-Weiss V (2014) *Hyalella cenotensis*, a new species of Hyalellidae (Crustacea: Amphipoda) from the Yucatán Peninsula, Mexico. Zootaxa 3811 (2): 262–270. https://doi.org/10.11646/zootaxa.3811.2.7

Marrón-Becerra A, Hermoso-Salazar M, Solis-Weiss V (2018) *Hyalella maya*, a new Hyalellidae species (Crustacea: Amphipoda) from a cenote in the Yucatan Peninsula, Mexico. Journal of Cave and Karst Studies 80 (2): 1–11. https://doi.org/10.4311/2017LSC0115

Nimer E (1989) Climatologia do Brasil. Instituto Brasileiro de Geografia e Estatística, Departamento de Recursos Naturais e Estudos Ambientais, Rio de Janeiro, Brazil, 421 pp.

Pereira VFGC (1989) Uma nova espécie de anfípode cavernícola do Brasil: *Hyalella caeca* sp. n. (Amphipoda, Hyalellidae). Revista Brasileira de Zoologia 6 (1): 49–55. https://doi.org/10.1590/S0101-81751989000100007

Persson LE (2001) Dispersal of *Platorchestia platensis* (Kroyer) (Amphipoda: Talitridae) along Swedish coasts: a slow but successful process. Estuarine, Coastal and Shelf Science 52 (2): 201–210. https://doi.org/10.1006/ecss.2000.0735

Reis GO, Penoni LR, Bueno AAP (2020) First record of the genus *Hyalella* (Amphipoda: Hyalellidae) from Santa Catarina state, Brazil, with description of two new species. Biota Neotropica 20 (2): e20190879. https://doi.org/10.1590/1676-0611-bn-2019-0879

Resende LPA, Zepon T, Bichuette ME, Pape RB, Gil-Santana H (2016) Associations between Emesinae heteropterans and spiders in limestone caves of Minas Gerais, southeastern Brazil. Neotropical Biology and Conservation 11 (3): 114–121. https://doi.org/10.4013/nbc.2016.113.01

Rodrigues SG, Bueno AAP, Ferreira RL (2012) The first hypothelminorheic Crustacea (Amphipoda, Dogielinotidae, *Hyalella*) from South America. ZooKeys 236: 65–80. https://doi.org/10.3897/zookeys.236.3930

Rodrigues SG, Bueno AAP, Ferreira, RL (2014) A new troglobitic species of *Hyalell*a (Crustacea, Amphipoda, Hyalellidae) with a taxonomic key for the Brazilian species. Zootaxa 3815 (2): 200–214. https://doi.org/10.11646/zootaxa.3815.2.2

Rodrigues SG, Senna AR, Quadra A, Bueno, AAP (2017) A new species of *Hyalella* (Crustacea: Amphipoda: Hyalellidae) from Itatiaia National Park, Brazil: an epigean freshwater amphipod with troglobiotic traits at 2,200 meters of altitude. Zootaxa. 4344 (1): 147–159. https://doi.org/10.11646/zootaxa.4344.1.6

Rogers DC, Magalhães C, Peralta M, Ribeiro FB, Bond-Buckup G, Price WW, Guerrero-Kommritz J, Mantelatto FL, Bueno A, Camacho AI, González ER, Jara CG. Pedraza M, Pedraza-Lara C, Latorre ER, Santos S (2020) Phylum Arthropoda: Crustacea: Malacostraca. In: Rogers DC, Damborenea C, Thorp J (Eds.) Thorp and Covich's freshwater invertebrates, volume 5: keys to Neotropical and Antarctic fauna. Fourth edition. Academic Press, Amerstam, the Netherlands, 809–986. https://doi.org/10.1016/B978-0-12-804225-0.00023-X

Rueden CT, Schindelin J, Hiner MC, DeZonia BE, Walter AE, Arena ET, Eliceiri KW (2017) ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18 (529): 1–26. https://doi.org/10.1186/s12859-017-1934-z

Ruffo S (1957) Una nuova specie troglobia di *Hyalella* del Venezuela. Estrato Dauli Annali del Museo Civico di Storia Naturale Di Genova 69: 363–369.

Secutti S, Bichuette ME (2013) Ictiofauna da área cárstica de Presidente Olegário, Estado de Minas Gerais, com ênfase nas espécies subterrâneas. Revista da Biologia 10 (2): 13–20. https://doi.org/10.7594/revbio.10.02.03

Simon KS, Benfield EF, Macko SA (2003) Food web structure and the role of epilithic biofilms in cave streams. Ecology 84 (9): 2395–2406. https://doi.org/10.1890/02-334

Trajano E (2012) Ecological classification of subterranean organisms. In: White WB, Culver DC (Eds.) Encyclopedia of caves. Academic Press, Waltham, USA, 275–277. https://doi.org/10.1016/B978-0-12-383832-2.00035-9

Trajano E (2013) Variações anuais e infra-anuais em ecossistemas subterrâneos: implicações para estudos ambientais e preservação de cavernas. Revista da Biologia 10 (1): 1–7. https://doi.org/10.7594/revbio.10.02.01

Väinölä R, Witt JDS, Grabowski M, Bradbury JH, Jazdzewski K, Sket B (2008) Global diversity of amphipods (Amphipoda; Crustacea) in freshwater. Hydrobiologia 595 (1): 241–255. https://doi.org/10.1007/s10750-007-9020-6

Vesper DJ (2012) Contamination of cave waters by heavy metals. In: White WB, Culver DC (Eds.) Encyclopedia of caves. Academic Press, Waltham, USA, 161–166. https://doi.org/10.1016/B978-0-12-814124-3.00035-2

Yule CM (2004) Crustacea, Peracarida, Amphipoda. In: Yule CM, Sen YH (Eds.) Freshwater invertebrates of the Malaysian region. Academy of Sciences Malaysia, Selangor, Malaysia, 307–311.

Zakšek V, Delić T, Fišer C, Jalžić B, Trontelj P (2019) Emergence of sympatry in a radiation of subterranean amphipods. Journal of Biogeography 46 (3): 657–669. https://doi.org/10.1111/jbi.13514

Zepon T, Bichuette ME (2017) Influence of substrate on the richness and composition of Neotropical cave fauna. Anais da Academia Brasileira de Ciências 89 (3): 1615–1628. https://doi.org/10.1590/0001-3765201720160452